REMARKS

I. Preliminary Remarks

The Examiner objected to claims 21-23 under 37 C.F.R. § 1.75(c) as being in improper multiple dependent form. Claims 21-23 are amended by the foregoing amendment to solely depend from claim 17. New claims 42-44 are identical to claims 21-23, but depend from claim 18 instead of claim 17. These amendments do not add new matter to the specification. In view of these amendments the objection to claims 21-23 should be withdrawn.

II. The rejection under 35 U.S.C. § 112, first paragraph for lack of enablement should be withdrawn.

The Examiner maintained the rejection of claims 1-10, 17-18, 21-23 and 25-26 under 35 U.S.C. § 112, first paragraph for lack of enablement. In particular, the Examiner stated that the specification does not reasonably provide enablement for any AAV vector other than AAV2 with insertions at corresponding sites. Applicants submitted a Declaration of Jeffrey Bartlett, Ph.D. under 37 C.F.R. § 1.132 (denoted as the "Declaration"), which the Examiner did not find persuasive. The following remarks traverse the Examiner's reasoning for not finding the evidence in the Declaration persuasive.

A. The Two-Dimensional Model of AAV

The Examiner stated that the usefulness of the two-dimensional model of AAV2 and other parvovirus VP3 proteins, as provided in the Declaration, is unclear. For one, the Examiner stated that the models were generated post-filing. Regardless of whether the computer models were generated post-filing, one of skill in the art could have predicted the two-dimensional structure of AAV capsid proteins at the time of filing as the structural analysis techniques were available. This is demonstrated in the specification at page 11, lines 12-22, and in the Declaration in paragraphs 3 and 5 Figure 1 in Wu *et al.*, (*J. Virol.* 74: 8635-6847, 2000), provided as Exhibit A, is further evidence that one of skill in the art could predict the secondary structure of an AAV capsid protein at the time of filing. This figure depicts a predicted secondary structure of an AAV capsid protein that is an adaptation of a comparison of parvovirus sequences generated by Chapman and Rossman (*Virol.* 194: 491-509, 1993). This paper provides another example of the available techniques for predicting secondary structure at the time of filing. In addition, the structure depicted in Figure 1 of Wu

et al. is similar to the two-dimensional model of AAV capsid proteins provided in the Declaration. The Examiner does not provide any evidence to doubt that the post-filing model could not have been generated at the time of filing.

The Examiner also stated that the two-dimensional model revealed that the location of multiple insertion sites of the invention was in a region that did not resolve well and therefore the usefulness of the model is unclear. The two-dimensional models, depicted in the Declaration, demonstrate that the modeled parvovirus capsid protein structures had an overall basic structure with a common area of variation and the disclosure in Wu *et al.* (Exhibit A) confirms the models presented in the Declaration. The goal of the invention is to insert target peptides into the capsid protein of AAV while retaining the conformation of the capsid protein, vector infectability and particle formation. Therefore, the variable regions within the capsid proteins were predicted to tolerate the insertions without disrupting the structure and function of the vector. The model demonstrates that the variable region (large surface exposed loops) are commonly located in all the parvovirus (include 5 AAVserotypes) capsid proteins modeled. Therefore, using the teachings in the specification, one of skill in the art can model the two-dimensional structure of the protein and insert the target within the variable region. Further, the experimental data provided in the Declaration demonstrated that making insertions in these loops, as predicted in the specification, do not alter the structure and function of the vector.

B. Support for the Teachings of the Declaration in the Specification

The Examiner also stated that the experimental evidence provided in the Declaration cannot be extrapolated to the present invention because the specification lacks support for these concepts. The identification of insertion sites in AAV1, AAV3, AAV4 and AAV5, as described in the Declaration, were based on comparisons of the primary and secondary structure of the AAV serotype using the knowledge of the insertion sites taught in the specification. IN particular, figures 3 and 4 of the Declaration compared the secondary structures of the AAV serotypes in order to identify the locations of the surface-exposed variable loops. The primary structures were then aligned using standard methods in the art to determine the corresponding location of the insertion sites taught in the specification to be effective in AAV2. The comparison of AAV2 and the known structure of other parvoviruses (Figs. 1 and 2 of the Declaration) substantiates the location of the loops exposed to the

surface of the AAV2 vector. One of skill in the art could easily do these alignments and determine the corresponding insertion site in the other AAV serotypes based on the teachings in the specification.

The Examiner states that the specification teaches the insertion sites in VP1 while the declaration teaches how to identify the insertion sites in VP3. The Examiner asserts that "the structure of VP1 is dissimilar to VP3 but is similar to VP2, " and cites Kronenberg *et al.* (*EMBO Reports* 2: 997-1002, 2001) as support for this statement. However, Kronenberg *et al.* and the specification (page 1, lines 25-28) teach that the three capsid proteins (VP1, VP2 and VP3) are generated by alternative splicing with nonconsensual start codons. These protein have a common C-terminal region with varying N-terminals. (See Kronenber *et al.*, page 997, col. 1, paragraph 2). Attached as Exhibit B is an alignment of the amino acid sequence of AAV2 VP1 (SEQ ID NO: 13), VP2 (SEQ ID NO: 14) and VP3 (SEQ ID NO: 15). This alignment demonstrates that these capsid proteins only differ in the N-terminus. It is standard in the art to experimentally manipulate VP3 as it is contained in all three structural proteins, for example see Figure 2 in Wu *et al.* (Exhibit A). The amino acid locations are defined by one of skill in the art by their location in VP1 in order for consistence throughout the three proteins.

Kronenberg *et al.* describes the investigation of empty AAV2 caspids by electron cryo-microscopy and iscosaherdral image reconstruction, which resulted in the three-dimensional mapping of the AAV2 capsid. The AAV2 map was then compared to other known parvoviruses, which showed the outer region of the AAV2 caspids differed significantly from other parvoviruses, as pointed out by the Examiner in the Action (page 7 of the Action). However, Kronenberg *et al.* supports the teachings in the specification by demonstrating that the location of two insertion sites taught by Girod *et al.* (*Nat. Med.* 5: 1052-1056, 1999) AAV2-1447 and AAV2-1587) on the three-dimensional AAV2 map matched well to the atomic model of canine parvovirus. The insertion sites of the present invention are only one away from those taught be Girod *et al.* thus, Kronenberg *et al.* supports the theory that the insertion sites in AAV2 are predictive of insertion sites in other AAV serotypes.

In summary, the working examples in the specification used the secondary structure of the AAV2 capsid protein to identify regions expected to be exposed to the

surface of the vector while being in a region that will not effect structure or function of the vector. The Declaration uses the techniques taught in the working examples of the specification and repeats the experiments in AAV1, AAV3, AAV4 and AAV5. The data in the Declaration demonstrates that the location of the AAV2 insertion sites can predict the corresponding insertion sites in other AAV serotypes. Identifying the insertion sites in the other AAV serotypes does not require undue experimentation as the guidance to predict the insertion sites is provided in the specification; and this is confirmed by the experiments described in the Declaration. Thus, claims 1-10, 17-18, 21-23 and 25-26 are enabled by the specification and the rejection under 35 U.S.C. § 112, first paragraph should be withdrawn.

III. The rejection under 35 U.S.C. § 112 for lack of adequate written description should be withdrawn.

The Examiner rejected claims 1-10, 17-18, 21-23 and 25-26 under 35 U.S.C. § 112, first paragraph for failing to comply with the written description requirement. In maintaining the rejection the Examiner stated

> Applicants teach that surface and secondary structural regions were identified in a comparison of five parvoviruses. However, this broad and hypothetical functional characteristic [of the claimed invention] was not used to identify the specific sites of AAV that could tolerate insertion mutations and provide for presentation of peptide for targeting or immunogen presentation. Rather site-directed mutagenesis of AAV2 was used to identify the specific sites of insertion was not used to identify the regions of insertions. (page 8 of the Action)

The invention is directed to insertion sites in the AAV capsid protein which do not affect the conformation of the capsid protein, vector infectability or particle formation. The specification teaches that these insertion sites can be identified by comparing the secondary structures of the capsid protein to known structure of other parvoviruses to determine the loops exposed to the surface of the vector. One of the inventive contributions of this invention is the teaching that the exterior loops will toleratethe insertion of targets or immunogens while retaining the structure and function of the vector. The working examples provide insertion sites in the AAV2 capsid protein, but due to the similarity of the secondary structure of the capsid proteins of the genus AAV, one of skill will understand that Applicants were in possession of the ability to make insertions in any AAV capsid protein.

Contrary to the Examiner's statements, the specification identifies regions of insertion in addition to the identification of specific sites within AAV2. The specification states that the invention is based on the eleucidation of regions in the AAV capsid that are amenable to insertion of heterologous proteins (see page 3, lines 22-26 and page 4, lines 8-12). The insertion sites must be in a region that is exposed to the surface of an AAV vector and does not disrupt confirmation of the capsid protein, infectability of the vector or particle formation (see page 5, lines 24-27). Further, the Declaration provides evidence that a standard alignment of the primary structure of caspid protein sequences allows one of skill to identify the corresponding insertion sites in any AAV serotype using the information disclosed in the specification.

Further, the specification teaches 20 specific insertion sites, which is a representative number of species of the claimed genus. The claims are directed to an AAV vector comprising a capsid protein with an insertion at a position corresponding to the recited position in VP1 of AAV2. These claims are not directed to any insertion site in any AAV serotype as the Examiner alleges; rather, the claims are directed to specific insertion sites that may be determined by a standard alignment of the caspid protein amino acid sequences of interest and the caspid protein of AAV2. As the amino acid sequences of the capsid protein of the AAV serotypes are known in the art, one of skill can readily identify the position of the corresponding insertion sites that are describes in the specification. Therefore, the specification provides adequate written description of the claimed genus and the rejection of claims 1-10, 17-18, 21-23 and 25-26 under 35 U.S.C. § 112, first paragraph for lack of adequate written description should be withdrawn.

CONCLUSION

This amendment and request for reconsideration is a filed with a Request for Continued Examination and should be considered the required submission under 37 C.F.R. § 1.114. Also enclosed is the fee due under 37 C.F.R. § 1.17(e). Thus, the Applicants respectfully request entry of the foregoing amendment. The Applicants believe claims 1-10, 17-18, 21-23 and 25-26 are in condition for allowance and request notification of the same. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejections of the claims and to pass this application to issue.

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Respectfully submitted,

By _____

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